Novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on pharmacokinetic property-driven optimization

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ABSTRACT

We previously reported a highly potent DPP-IV inhibitor **6** with low in vivo efficacy. While trying to maintain consistent in vitro and in vivo biological activity, we initiated a pharmacokinetic property-driven optimization to improve the metabolic stability and permeability of inhibitor **6**. A simple scaffold replacement of thienopyrimidine with pyrrolopyrimidine (**21a**) led to significantly improved metabolic stability (4% vs. 65% remaining). Further modification of the pyrrolopyrimidine scaffold to produce compound **21j** resulted in much better oral bioavailability than **6**. Importantly, compound **21j** exhibits greater in vivo efficacy than does **6** and Alogliptin and is worthy of further development.

Keywords: DPP-IV inhibitor; type 2 diabetes; PK-driven optimization; pyrrolopyrimidine analogues;

oral glucose tolerance test.

1. Introduction

With more than 220 million people affected worldwide, diabetes has emerged in this century as an epidemic. Type 2 diabetes (T2D) formerly referred to as non-insulin-dependent or adult-onset diabetes, results from the body's ineffective use of insulin and comprises 90% of people who have diabetes. The growing number of deaths attributable to diabetes reflects the insufficient glycemic control achieved by past and current treatments [1]. Since 2004, dipeptidyl peptidase IV (DPP-IV) inhibitors have been demonstrated to be effective and safe compounds that control blood glucose for good patient compliance and reduced risks of hypoglycemia and other side effects [2-4]. To date, Sitagliptin **1** [5-6], Vildaglipin **2** [7-9], Saxagliptin **3** [10], Alogliptin **4** [11] and Linagliptin **5** [12] are on the market in many countries (Figure 1).

We previously reported a novel series of DPP-IV inhibitors [13]. Compound **6** (IC_{50}=0.33 nM) was observed to be 10 times more potent against DPP-IV than Alogliptin. Furthermore, this compound was selective for DPP-IV over other enzymes within the dipeptidyl peptidase (DPP) family. However, the in vivo efficacy of compound **6** is similar to that of Alogliptin. Therefore, we reasoned that the insufficient in vivo activity might due to the poor pharmacokinetic properties of compound **6** for its low bioavailability.

In this study, we conducted stability studies of thienopyrimidine analogues followed by the modification of the scaffold to determine the disconnection between in vitro and in vivo activities. All of the reported thienopyrimidine analogues suffer from severe hepatic clearance in metabolic stability studies. Surprisingly, a simple scaffold replacement with the isostere pyrrolopyrimidine significantly improved the percentage remaining from 4% to 65%. However, compound **21a** lost oral bioavailability (5.7 %), due to its poor absorption caused by poor permeability. Various substituents were attached to the ring of compound **21a** to increase the permeability and to reduce the transporter-mediated efflux to

avoid low oral bioavailability. As a result, we generated a new series of highly potent and selective DPP-IV inhibitors, as represented by compound **21j**, which has a good balance of activity, selectivity, PK properties and in vivo efficacy. In this study, we report how an anti-diabetic preclinical drug candidate was generated from the step-by-step process of the PK-driven optimization of a novel DPP-IV inhibitor.

2. Chemistry

The synthesis of compounds **21a**–**d** is outlined in Scheme 1. Briefly, 6-Methyluracil **11** was easily transformed to **12** via a typical nitrification. Subsequently, **13** was obtained via a condensation reaction of **12** and N, N-dimethylformamide dimethyl acetal, then **13** was treated by zinc powder in acetic acid (AcOH) to give the intermediate **17c** in overall 20.3% yield [14]. Commercially available 6-aminopyrimidine-2, 4(1H, 3H)-dione **14** reacted with 1-chloro-2-propanone to give compound **17b** in 80% yield. **17a** was synthesized by heating commercially available ethyl 2-cyano-4, 4-diethoxybutanoate **15** with urea at 80 °C. The resulting reaction mixture was subsequently acidified with concentrated HCl to afford the diol compound **17a** with the yield of 92.1% [15]. 2, 4-Dichloropyrrolopyrimidines **18a-c** were readily synthesized from **17a**–**c** by chlorination with $POCl_3$. Selective hydrolysis with sodium hydroxide (NaOH) gave the chloropyrrolopyrimidines **19a**–**c** [16]. **19d** was obtained using the same conditions from the N-methyl 2,4-Dichloropyrrolopyrimidines **18d**, which was synthesized by a classical methylation [17]. **19a**–**c** were N-protected by tert-butyloxycarbonyl (Boc) protecting groups [18] to give **19a'**–**c'**. Alkylation of **19a'**–**c'** and **19d** with 2-cyanobenzyl bromide provided **20a-d** [19]. Finally, compounds **21a**–**d** were generated from compound **20a**–**d** by chloride displacement with 3-(R)-aminopiperidine with yields of 67.3%-80% [13].

The synthesis of **21e**–**i** and **21j**–**m** were outlined in Scheme 2 and Scheme 3 respectively. The bromo compounds **21e**, **21f** and **21j** were obtained by bromination of corresponding N-protected pyrrolopyrimidines **22d**, **22f** and **22j** respectively [20], **22f** and **22j** also served as intermediates for the preparation of aryl substitute pyrrolopyrimidines. Desired aryl compounds **21g**–**i** and **21k-m** were made

mainly using conventional Suzuki coupling conditions in yields of 30%-45% [21].

3. Results and Discussion

3.1. Metabolic stability study on thienopyrimidine analogues.

The highly potent inhibitor **6** (IC_{50}=0.33 nM), which is 10 times more potent than Alogliptin, displayed a similar anti-diabetic effect to Alogliptin in an oral glucose tolerance test (OGTT) [13]. We assumed that the disconnection between in vitro and in vivo activity was due to the poor pharmacokinetic (PK) properties (23.3% oral bioavailability in rats) of compound **6**. To better understand the PK profiles of this compound, we tested the hepatic first pass effect. Not surprisingly, compound **6** displayed a 93% turnover after 30 minutes incubation in pooled rat liver microsomes (RLM) (Table 1). The modifications of adding a methyl group, rotating the ring and substitution with trifluoromethyl did not significantly change the hepatic first pass effect. All of the reported thienopyrimidine analogues (compounds **7-10**, Table 1) suffer from severe metabolic instability. We reasoned that the thienyl ring might be a fragile functional group that needed to be replaced in order to increase the metabolism stability.

3.2. Improvement of metabolic stability.

We considered replacing the thienyl ring to reduce the hepatic first pass with a more stable isostere. Thus, we decided to replace thienopyrimidine with pyrrolopyrimidine. Notably, compound **21a** was observed to exhibit good metabolism stability as the percentage remaining in the RLM improved to 65.07% after 30 minutes incubation (Table 2).

However, further evaluation of its PK property showed that compound **21a** did have significantly lower oral bioavailability (5.7%). Because oral bioavailability is directly related with not only hepatic first pass effect but also absorption, we realized that compound **21a** might have a poor absorption. The placement of the pyrrolopyrimidine scaffold seemed to successfully avoid the hepatic first pass effect while introducing poor absorption.

3.3. Improvement of absorption profile.

The cascade of events determining oral bioavailability is well-known today [22-25]. Permeability is directly related with drug absorption and oral bioavailability [26-29]. The caco-2 permeability bidirectional study was used to measure the permeability of compound **21a** (Table 2), which was determined to exhibit notably low permeability from the apical side (A) to the basolateral side (B). Furthermore, a high transporter mediated efflux from B to A. Thus the efflux ratio of compound **21a** is sufficiently high (19.0) and leads to poor absorption.

To date, there are no direct structure-function relationships or pharmacophore models to describe the structural features responsible for transporter substrates. Thus, we adopted several simple strategies based on the rules described in the literature [30-33] to improve the absorption and reduce transporter-mediated efflux, which led to the synthesis of a series of pyrrolopyrimidine analogues.

The addition of substituents to the ring was sufficiently effective that the incorporation of 2-methyl (compound **21b**) and 2-bromo (compound **21f**) immediately reduced the efflux ratio (2.15 and 2.08). These data suggest that creating some steric hindrance may reduce the interaction of the compound with transporters. Incorporating alkyl groups could also increase the lipophilicity of compounds, improving their permeability to a certain extent.

Encouraged by compound **21f**, we continued to add aromatic groups (compounds **21g-i**) and evaluated the absorption of these compounds. Only compound **21i** with the pyridyl ring exhibited the desired score, whereas the other analogues, **21g** and **21h**, lost permeability. The blockage of the 1-nitrogen with 1-methyl (compound **21d**), in order to reduce the H-bond donor, greatly increased the ratio to 13.3. However, the addition of two bromo groups to **21d** (compound **21e**) dropped the ratio to a reasonable number (3.37) and improved its permeability.

The process between compounds and transporters is a configuration interaction. The rotated analogues (compounds **21c**, and **21j-m,** Table 3) were synthesized and evaluated for their permeability. The unsubstituted compound **21c** acquires much better permeability compared with its isomer **21a** (4.20 vs. 19.0, respectively). Further modification of **21c** produces the compound **21j** and **21l** with a low efflux

ratio (6.55 and 2.51, respectively).

3.4. Determination of the metabolic stability of key compounds

All of the analogues with efflux ratios less than 10 were further evaluated for their hepatic stability (Table 2 and Table 3). Compound **21e**, **21c** and **21l** may induce hepatic metabolism because the percentage remaining is less than 50%. Therefore, biological evaluations were performed on compounds **21b**, **21f**, **21i** and **21j**.

3.5. In vitro biological evaluations.

Through PK-driven optimization, four of the compounds (**21b**, **21f**, **21i** and **21j**) were selected for their acceptable in vitro PK properties. These compounds were subsequently moved forward to evaluate their activity against DPP-IV and selectivity against DPP-8 and DPP-9 using Alogliptin as a reference.

Fortunately, all of the compounds (Table 4) were observed to be potent inhibitors against the DPP-IV enzyme with single-digit nanomolar IC_{50} values with the exception of compound **21b**. In our studies, compounds **21f**, **21i** and **21j** were observed to be more active than Alogliptin in vitro. The IC_{50} value measure with Alogliptin in our study is 3.4 nM, which is in accordance with the literature value (IC_{50}< 10 nM) [11]. Additionally, all the compounds were observed to exhibit more than a 1000-fold DPP-IV selectivity over DPP-8 and DPP-9. The compounds also did not inhibit the human Ether-à-go-go Related Gene (hERG) ion channel, which may be related with cardiovascular risk [34-35].

Compound **21j** was then confirmed to be the most balanced inhibitor with both in vitro PK properties and activity of the synthesized analogues.

3.6. In vivo pharmacokinetic and pharmacodynamic evaluations.

Based on the in vitro PK data, potency, selectivity and lack of hERG inhibition, compound **21j** was moved to in vivo studies. In rat, **21j** shows a 41% oral bioavailability with a half life around 2 hours, similar to that of Alogliptin in rat (BA= 45%) [36]. Considering results of compound **6** and Alogliptin, **21j** could probably behave better in dogs or monkeys.

In the following OGTT in mice, compound **21j** was shown to be effective to reduce glucose excursion.

All of the data presented in Figure 2 clearly demonstrate that compound **21j** is superior to compound **6** and Alogliptin in C57BL/6j lean mice at a dosage of 3 mg/kg. An in-depth evaluation in ob/ob mice revealed that compound **21j** displayed a significantly reduced glucose excursion in a dose-dependent manner from 1 mg/kg (27.9% inhibition), 3 mg/kg (51.9% inhibition) to 10 mg/kg (72.7% inhibition).

Compound **21j** was demonstrated to be a potent (IC_{50} =1.40 nM) inhibitor of DPP-IV and exhibits great selectivity over DPP-8 and DPP-9. Additionally, this compound exhibited a fine oral bioavailability and reduced glucose excursion in a dose-dependent manner in T2D model mice. Compared with compound **6** and Alogliptin, compound **21j** has greater in vivo efficacy.

4. Conclusions

In summary, further pharmacokinetic evaluations on our recently reported DPP-IV inhibitors demonstrated a comprehensive hepatic instability within the thienopyrimidine scaffold. Scaffold replacement with pyrrolopyrimidine (compound **21a)** improved the metabolic stability from 6.6% to 65% of the remaining parent in RLM, while resulting in poor absorption. Structural modification of the pyrrolyl ring led to a series of analogues with significantly improved permeability, as reflected by the efflux ratios (19.0 v.s. 2.08 – 6.55). Four compounds (**21b**, **21f**, **21i** and **21j**) were observed to have acceptable in vitro PK properties (percentage remaining > 50%, efflux ratio < 10), high in vitro activity within the nM range, high selectivity over DPP-8 and DPP-9 and lack of hERG inhibition.

The most active compound **21j** was identified as a promising preclinical candidate with fine oral bioavailability and superior in vivo efficacy, making this compound worth further development. Notably, our results validate that PK-driven optimization is an efficient way to modify highly potent compounds with poor PK properties toward the generation of desired preclinical drug candidates.

5. Experimental Section

5.1. Chemistry.

All commercially available compounds and solvents were reagent grade and were used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang

GF_{254} silica gel plates (5 x 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) and by spraying with an ethanol solution of 2,4-DNP or Ninhydrin or by being fumed with iodine steam. Silica gel column chromatography was performed on silica gel (200–300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker NMR AVANCE 500 (500 MHz). Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). MS data were measured on an Agilent MSD-1200 ESI-MS system. Purity data were acquired on a Gilson LC/UV system using a Phenomenex C18 with a 150 mm × 4.6 mm column and a 5 μ particle size. The following gradients (A, 0.1% TFA in H2O; B, 0.1% TFA in MeOH) were used for 30 min at a flow rate of 1 mL/min at 25 ℃: a 45% B isogradient was used for compounds **21a**, **21f**, **21h** and **21l-m**; a 58% B isogradient was used for compounds **21b-e**, **21g**, and **21i-k**. All test compounds were confirmed to be ≥95% pure.

*5.2. (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl) benzonitrile (**21a**).*

A mixture of **20a** (13.1 g, 43.4 mmol), 3-(*R*)-aminopiperidine dihydrochloride (11.5 g, 66.0 mmol) and $NaHCO_3$ (17.4 g, 173.6 mmol) in a sealed tube containing 300 mL of ethanol was heated at 150 ℃ for 6 hours. The reaction mixture was subsequently cooled to room temperature and filtered. The resulting filtrate was concentrated in vacuo and then purified by flash chromatography to yield compound **21a** as white powder. Yield: 67.3%. ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 7.64 (1H, d, *J*= 6.8 Hz), 7.45 (1H, t, *J*= 7.6 Hz), 7.31 (1H, t, *J*= 7.2 Hz), 7.07 (1H, d, *J*= 8.0 Hz), 6.81 (1H, d, *J*= 3.2 Hz), 6.64 (1H, d, *J*= 3.2 Hz), 5.55 (2H, AB q, *J*= 38.8 Hz, 16.0 Hz), 3.21-3.17 (1H, m), 3.05-3.02 (2H, m), 2.82-2.77 (2H, m), 1.94-1.90 (1H, m), 1.73-1.68 (1H, m), 1.63-1.55 (1H, m), 1.45-1.43 (1H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 22.83 (1C), 31.49 (1C), 45.41 (1C), 47.11 (1C), 51.34 (1C), 56.96 (1C), 102.17 (1C), 104.02 (1C), 110.46 (1C), 116.98 (1C), 120.19 (1C), 126.69 (1C), 127.47 (1C), 132.72 (1C), 133.01 (1C), 141.66 (1C), 147.45 (1C), 155.75 (1C), 161.00 (1C). ESI-MS calculated for

($C_{19}H_{20}N_6O$) $[M+H]^+$, 349.2, found 349.1.

*5.3. (R)-2-((2-(3-aminopiperidin-1-yl)-6-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl) methyl)benzonitrile (**21b**).*

Compound **21b** was prepared in a manner identical to that described for **21a** as yellow powder. Yield: 72%. ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 7.63 (1H, d, *J*= 7.2 Hz), 7.44-7.40 (1H, m), 7.31-7.29 (1H, m), 7.01 (1H, d, *J*= 7.6 Hz), 6.28 (1H, s), 5.55 (2H, AB q, *J*= 24.0 Hz, 16.0 Hz), 3.15-3.12 (1H, m), 2.98-2.95 (2H, m), 2.73-2.71 (2H, m), 2.30 (3H, s), 1.91-1.89 (1H, m), 1.76-1.66 (1H, m), 1.59-1.57 (1H, m), 1.40-1.29 (1H, m). ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 15.33 (1C), 24.86 (1C), 30.99 (1C), 45.78 (1C), 47.83 (1C), 51.65 (1C), 57.31 (1C), 102.55 (1C), 105.02 (1C), 111.54 (1C), 118.98 (1C), 125.17 (1C), 127.01 (1C), 127.91 (1C), 132.77 (1C), 133.24 (1C), 141.89 (1C), 147.90 (1C), 155.50 (1C) , 161.58 (1C). ESI-MS calculated for ($C_{20}H_{22}N_6O$) $[M+H]^+$, 363.2, found 363.2.

*5.4. (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl) benzonitrile (**21c**).*

Compound **21c** was prepared from 2-((2-chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d] pyrimidin-3-yl)methyl)benzonitrile **20c** by a method similar to that used to make **21a** as white powder. Yield: 75%. ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 7.66 (1H, dd, *J*= 7.6 Hz, 0.8 Hz), 7.45-7.41 (1H, m), 7.31 (1H, t, *J*= 7.6 Hz), 7.24 (1H, d, *J*= 2.8 Hz), 6.93 (1H, d, *J*= 8.0 Hz), 6.41 (1H, d, *J*= 2.8 Hz), 5.62 (2H, s), 3.17-3.14 (1H, m), 3.00-2.90 (2H, m), 2.81-2.76 (1H, m), 2.68-2.63 (1H, m), 1.93-1.89 (1H, m), 1.76-1.72 (1H, m), 1.63-1.55 (1H, m), 1.24-1.23 (1H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 22.39 (1C), 29.83 (1C), 45.05 (1C), 47.17 (1C), 51.35 (1C), 55.05 (1C), 102.53 (1C), 110.44 (1C), 115.25 (1C), 117.03 (1C), 126.69 (1C), 127.56 (1C), 128.54 (1C), 132.78 (1C), 133.11 (1C), 141.55 (1C), 143.28 (1C), 153.94 (1C), 155.58 (1C). ESI-MS calculated for ($C_{19}H_{20}N_6O$) $[M+H]^+$, 349.2, found 349.1.

*5.5. (R)-2-((2-(3-aminopiperidin-1-yl)-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl) methyl)benzonitrile (**21d**).*

Compound **21d** was prepared in a manner identical to that described for **21a** as yellow powder. Yield: 80%. ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 7.66 (1H, dd, *J*= 7.6 Hz, 0.8 Hz), 7.48-7.44 (1H, m), 7.33-7.30 (1H, m), 7.02 (1H, d, *J*= 8.0 Hz), 6.74 (1H, d, *J*= 3.2 Hz), 6.62 (1H, d, *J*= 3.6 Hz), 5.57 (2H, s), 3.72 (3H, s), 3.17-3.14 (1H, m), 3.03-3.00 (1H, m), 2.97-2.91 (1H, m), 2.79-2.73 (1H, m), 2.63-2.57 (1H, m), 1.95-1.90 (1H, m), 1.77-1.72 (1H, m), 1.69-1.62 (2H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 23.01 (1C), 29.98 (1C), 32.26 (1C), 45.42 (1C), 47.11 (1C), 51.33 (1C), 57.80 (1C), 101.55 (1C), 103.91 (1C), 110.49 (1C), 116.93 (1C), 124.32 (1C), 126.62 (1C), 127.44 (1C), 132.71 (1C), 132.97 (1C), 141.66 (1C), 147.00 (1C), 155.86 (1C), 160.87 (1C). ESI-MS calculated for ($C_{20}H_{22}N_6O$) $[M+H]^+$, 363.2, found 363.2.

*5.6. (R)-2-((2-(3-aminopiperidin-1-yl)-5,6-dibromo-7-methyl-4-oxo-4H-pyrrolo[2,3-d] pyrimidin-3 (7H)-yl)methyl)benzonitrile (**21e**).*

Compound **21e** was prepared in a manner identical to that described for **21f** as white powder. Yeild: 88%. ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 7.65 (1H, d, *J*= 7.6 Hz), 7.49 (1H, t, *J*= 7.2 Hz), 7.33 (1H, t, *J*= 7.6 Hz), 7.07 (1H, d, *J*= 8.0 Hz), 5.50 (2H, s), 3.71 (3H, s), 3.21-3.19 (1H, m), 3.08-3.05 (1H, m), 2.97-2.92 (1H, m), 2.79-2.74 (1H, m), 2.62-2.57 (1H, m), 1.96-1.92 (1H, m), 1.77-1.72 (1H, m), 1.69-1.63 (2H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 23.06 (1C), 33.22 (1C), 32.35 (1C), 45.97 (1C), 47.07 (1C), 51.10 (1C), 57.66 (1C), 92.53 (1C), 102.81 (1C), 109.12 (1C), 110.45 (1C), 116.97 (1C), 126.92 (1C), 127.67 (1C), 132.91 (1C), 133.16 (1C), 141.29 (1C), 146.73 (1C), 156.85 (1C), 158.30 (1C). ESI-MS calculated for ($C_{20}H_{20}Br_2N_6O$) $[M+H]^+$, 521.0, found 521.0.

*5.7. (R)-2-((2-(3-aminopiperidin-1-yl)-6-bromo-4-oxo-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl)methyl) benzonitrile (**21f**).*

Compound **22a** (100 mg) was dissolved in DCM (20 mL), and then treated with NBS (31 mg). The mixture was stirred for 4 h at rt, and the solvent was removed under reduced pressure to yield a crude residue, which was purified by silica gel chromatography (DCM) to give the product (R)-tert-butyl(1-(6-bromo-3-(2-cyanobenzyl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin

-2-yl)piperidin-3-yl)carbamate (**22f**). To a solution of **22f** in DCM, TFA was added at 0 ℃. After being stirred at room temperature for 3 h, the solution was subsequently treated with saturated $NaHCO_3$ to pH 6-7. Subsequent removal of the solvent under reduced pressure gave a crude residue, which was purified by silica gel chromatography (DCM/MeOH) to give compound **21f** as grey powder. Yield: 92%. ^{1}H-NMR (400MHz, MeOD) δ ppm: 7.72 (1H, d, *J*= 7.2 Hz), 7.58 (1H, t, *J*= 7.6 Hz), 7.42 (1H, t, , *J*= 7.6 Hz), 7.18 (1H, d, *J*= 8.0 Hz), 6.84 (1H, s), 5.53 (2H, AB q, *J*= 34.4 Hz, 15.2 Hz), 3.54-3.44 (2H, m), 3.15-3.06 (2H, m), 2.92-2.87 (1H, m), 2.66-2.09 (1H, m), 1.88-1.84 (1H, m), 1.80-1.71 (1H, m), 1.65-1.55 (1H, m). ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 22.26 (1C), 27.93 (1C), 46.03 (1C), 46.94 (1C), 51.89 (1C), 52.80 (1C), 103.02 (1C), 104.65 (1C), 105.96 (1C), 110.61 (1C), 117.80 (1C), 127.67 (1C), 128.19 (1C), 133.40 (1C), 133.81 (1C), 142.11 (1C), 147.63 (1C), 156.22 (1C), 158.05 (1C). ESI-MS calculated for ($C_{19}H_{27}BrN_6O$) $[M+H]^+$, 427.1, found 427.0.

The synthesis of compounds **21g-i** and **21k-m** were made from Suzuki reaction. Tert-butyloxycarbonyl (Boc) deprotection was conducted according to the general procedure introduced in supplementary materials

*5.8. (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-6-(4-(trifluoromethyl)phenyl)-4H-pyrrolo[2,3-d] pyrimidin -3(7H)-yl)methyl)benzonitrile (**21g**).*

Yellow powder. Yield: 40.7%. ^{1}H-NMR (400MHz, MeOD) δ ppm: 7.87-7.81 (3H, m), 7.74-7.67 (3H, m), 7.43 (1H, t, *J*= 7.6 Hz), 7.22 (1H, d, *J*= 8.0 Hz), 6.96 (1H, s), 5.56 (2H, AB q, *J*= 34.8 Hz, 15.2 Hz), 3.61-3.58 (1H, m), 3.51-3.49 (1H, m), 3.19-3.14 (2H, m), 2.98-2.92 (1H, m), 2.15-2.13 (1H, m), 1.92-1.81 (1H, m), 1.80-1.78 (1H, m), 1.66-1.64 (1H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 21.84 (1C), 27.73 (1C), 46.06 (1C), 47.06 (1C), 51.42 (1C), 52.25 (1C), 100.85 (1C), 105.69 (1C), 110.51 (1C), 117.12 (1C), 122.93 (1C), 123.65 (1C), 124.63 (2C), 125.46 (1C), 125.49 (1C), 127.56 (1C), 127.71 (1C), 132.86 (1C), 133.07 (1C), 133.66 (1C), 141.31 (1C), 148.68 (2C), 155.85 (1C), 160.56 (1C). ESI-MS calculated for ($C_{26}H_{23}F_3N_6O$) $[M+H]^+$, 493.2, found 493.2.

*5.9. (R)-2-((2-(3-aminopiperidin-1-yl)-6-(4-fluorophenyl)-4-oxo-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl) methyl)benzonitrile (**21h**).*

Yellow powder. Yield: 41.2%. ^{1}H-NMR (400MHz, MeOD) δ ppm: 7.74-7.67 (3H, m), 7.58 (1H, t, *J*= 7.6 Hz), 7.42 (1H, t, *J*= 7.6 Hz), 7.19 (1H, d, *J*= 8.0 Hz), 7.13 (2H, t, *J*= 8.8 Hz), 6.76 (1H, s), 5.56 (2H, AB q, *J*= 37.2 Hz, 15.6 Hz), 3.58-3.55 (1H, m), 3.51-3.45 (1H, m), 3.18-3.13 (2H, m), 2.96-2.91 (1H, m), 2.15-2.12 (1H, m), 1.91-1.82 (1H, m), 1.78-1.73 (1H, m), 1.66-1.64 (1H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 21.78 (1C), 27.69 (1C), 45.89 (1C), 47.04 (1C), 51.39 (1C), 52.26 (1C), 105.54 (1C), 110.51 (1C), 115.24 (2C), 115.46 (2C), 117.13 (1C), 126.47 (1C), 127.41 (1C), 127.66 (2C), 132.83 (1C), 133.07 (1C), 133.67 (1C), 141.37 (2C), 148.20 (1C), 155.28 (1C), 160.55 (1C). ESI-MS calculated for ($C_{25}H_{23}FN_6O$) $[M+H]^+$, 442.2, found 442.2.

*5.10. 2-((2-((R)-3-aminopiperidin-1-yl)-4-oxo-6-(pyridin-3-yl)-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl) methyl)benzonitrile (**21i**).*

Yellow powder. Yield: 30%. ^{1}H-NMR (400MHz, MeOD) δ ppm: 9.21 (1H, s), 8.87 (1H, d, *J*= 8.0 Hz), 8.69 (1H, d, *J*= 5.2 Hz), 8.12-8.09 (1H, m), 7.72 (1H, d, *J*= 7.6 Hz), 7.61 (1H, t, *J*= 7.2 Hz), 7.43 (1H, t, *J*= 8.0 Hz), 7.30-7.26 (2H, m), 5.54 (2H, AB q, *J*= 35.6 Hz, 15.2 Hz), 3.71-3.65 (1H, m), 3.55-3.52 (1H, m), 3.27-3.18 (2H, m), 3.03-2.97 (1H, m), 2.17-2.14 (1H, m), 1.95-1.86 (1H, m), 1.81-1.79 (1H, m), 1.69-1.64 (1H, m). ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 22.57 (1C), 27.89 (1C), 47.36 (1C), 47.52 (1C), 52.09 (1C), 52.51 (1C), 105.19 (1C), 106.21 (1C), 110.16 (1C), 118.31 (1C), 125.08 (1C), 127.30 (1C), 128.90 (1C), 129.18 (1C), 130.64 (1C), 134.14 (1C), 134.54 (1C), 137.48 (1C), 139.65 (1C), 141.22 (1C), 141.66 (1C) , 149.86 (1C), 157.66 (1C), 160.27 (1C). ESI-MS calculated for ($C_{24}H_{23}N_7O$) $[M+H]^+$, 426.2, found 426.1.

*5.11. (R)-2-((2-(3-aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl) methyl)benzonitrile (**21j**).*

Compound **21j** was prepared from (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H -pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile **21c** by a method similar to that used to make **21f** as

white powder. Yield: 82%. ^{1}H-NMR (400MHz, MeOD) δ ppm: 8.05 (1H, d, *J*= 7.6 Hz), 7.88 (1H, t, *J*= 7.6 Hz), 7.74-7.71 (2H, m), 7.41 (1H, d, *J*= 8.0 Hz) , 5.82 (2H, AB q, *J*= 57.2 Hz, 15.6 Hz), 3.78-3.75 (1H, m), 3.72-3.64 (1H, m), 3.46-3.38 (2H, m), 3.21-3.18 (1H, m), 2.37-2.35 (1H, m), 2.14-2.11 (1H, m), 1.98-1.90 (2H, m). ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 22.35 (1C), 27.90 (1C), 45.69 (1C), 47.02 (1C), 49.05 (1C), 52.92 (1C), 90.20 (1C), 110.73 (1C), 115.77 (1C), 117.78 (1C), 127.49 (1C), 128.23 (1C), 128.31 (1C), 133.36 (1C), 133.93 (1C), 140.19 (1C), 141.93 (1C), 154.70 (1C), 154.76 (1C). ESI-MS calculated for ($C_{19}H_{19}BrN_6O$) $[M+H]^+$, 427.1, found 427.0.

*5.12. 2-((2-((R)-3-aminopiperidin-1-yl)-4-oxo-7-(pyridin-3-yl)-4H-pyrrolo[3,2-d]pyrimidin-3(5H)-yl) methyl)benzonitrile (**21k**).*

White powder. Yield: 45.2%. ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 9.55 (1H, s), 9.11 (1H, d, *J*= 8.4 Hz), 8.70 (1H, d, *J*= 5.6 Hz), 8.28 (1H, d, *J*= 3.2 Hz), 8.01 (1H, t, *J*= 6.4 Hz), 7.84 (1H, d, *J*= 7.6 Hz), 7.61 (1H, t, *J*= 7.6 Hz), 7.45 (1H, t, *J*= 7.6 Hz), 7.12 (1H, d, *J*= 8.0 Hz), 5.48 (2H, AB q, *J*= 29.6 Hz, 15.6 Hz), 3.60-3.57 (2H, m), 3.13-3.10 (2H, m), 3.00-2.90 (1H, m), 2.04-1.96 (1H, m), 1.90-1.81 (1H, m), 1.66-1.52 (2H, m). ^{13}C-NMR (125 MHz, MeOD) δ ppm: 21.58 (1C), 21.17c (1C), 46.99 (1C), 47.09 (1C), 51.76 (1C), 51.99 (1C), 109.00 (1C), 109.21 (1C), 116.41 (1C), 117.82 (1C), 127.16 (1C), 127.99 (1C), 128.10 (1C), 128.42 (1C), 133.67 (1C), 133.73 (1C), 133.76 (1C), 137.79 (1C), 137.42 (1C), 140.46 (1C), 140.80 (1C), 142.02 (1C), 155.31 (1C), 156.01 (1C). ESI-MS calculated for ($C_{24}H_{23}Br_2N_7O$) $[M+H]^+$, 426.2, found 426.2.

*5.13. (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-7-(pyridin-4-yl)-4H-pyrrolo[3,2-d]pyrimidin-3(5H)-yl) methyl)benzonitrile (**21l**).*

White powder. Yield: 42.5%. ^{1}H-NMR (400MHz, MeOD) δ ppm: 8.87-8.66 (2H, m), 8.62-8.51 (2H, m), 8.25 (1H, s), 7.64 (1H, d, *J*= 8.0 Hz), 7.50 (1H, t, *J*= 7.6 Hz), 7.34 (1H, t, *J*= 7.6 Hz), 7.15 (1H, d, *J*= 7.6 Hz), 5.51 (2H, AB q, *J*= 35.2 Hz, 15.6 Hz), 3.61-3.58 (1H, m), 3.43-3.39 (1H, m), 3.17-3.11 (2H, m), 2.91-2.85 (1H, m), 2.09-2.07 (1H, m), 1.84-1.81 (1H, m), 1.75-1.70 (1H, m), 1.61-1.58 (1H, m). ^{13}C-NMR (125 MHz, MeOD) δ ppm: 23.44 (1C), 28.99 (1C), 35.29 (1C), 47.40 (1C), 53.04 (1C), 53.81

(1C), 111.77 (1C), 112.65 (1C), 118.36 (1C), 119.08 (1C), 122.75 (1C), 128.97 (1C), 129.14 (1C), 132.04 (1C), 134.23 (2C), 134.40 (2C), 141.69 (1C), 142.07 (1C), 143.37 (1C), 153.50 (1C), 156.44 (1C), 157.00 (1C). ESI-MS calculated for ($C_{24}H_{23}Br_2N_7O$) $[M+H]^+$, 426.2, found 426.2.

5.14. 2-((2-((R)-3-aminopiperidin-1-yl)-4-oxo-7-(thiophen-2-yl)-4H-pyrrolo[3,2-d]pyrimidin-3(5H)-yl) methyl)benzonitrile ***(21m)****.*

Yellow powder. Yield: 38.6 %. ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 7.98 (1H, s), 7.82-7.78 (2H, m), 7.69 (1H, d, *J*= 4.0 Hz), 7.61-7.55 (3H, m), 7.06 (1H, d, *J*= 8.0 Hz), 5.46 (2H, AB q, *J*= 50.4 Hz, 15.6 Hz), 3.46-3.43 (1H, m), 3.36-3.33 (1H, m), 3.11-3.05 (2H, m), 2.90-2.75 (1H, m), 2.02-1.92 (1H, m), 1.88-1.76 (1H, m), 1.66-1.46 (2H, m). ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 30.00 (1C), 35.43 (1C), 46.66 (1C), 47.69 (1C), 52.56 (1C), 53.21 (1C), 110.76 (1C), 113.58 (1C), 116.58 (1C), 118.56 (1C), 126.67 (1C), 127.09 (1C), 128.84 (1C), 129.11 (1C), 129.93 (1C), 130.02 (1C), 134.20 (1C), 134.52 (1C), 134.59 (1C), 140.49 (1C), 142.27 (1C), 154.90 (1C), 155.90 (1C). ESI-MS calculated for ($C_{23}H_{22}$N6OS) $[M+H]^+$, 431.2, found 431.2.

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